PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
4239-54106		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 00/06946	16/03/2000	16/03/1999

Applicant

THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[X] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[X] furnished subsequently to this Authority in computer readble form.

[X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 4

[ ] as suggested by the applicant.

[X] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/06946

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 47,50,52-55 (completely) and claims 44-46 (partially) are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 38, 51
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 38, 51

Present claims 38 and 51 relate to a product defined by reference to a desirable characteristic or property, namely a nucleic acid encoding all bispecific fusion proteins or all protein analogs, derivatives or mimetics being capable of binding to two sites on a target protein wherein the first binding domain binds to an inducing site on the target protein and the second binding site neutralises said induced epitope by forming a complex. The two domains are linked by a linker.

The claims cover all products having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such products. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/06946

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K16/10 C07K16/42 C07K14/705 C07K14/715 C07K19/00
C12N15/62 C12N5/10 A61K39/42 A61K38/17 A61P31/18
//C07K16/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, WPI Data, PAJ, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 47318 A (PROGENICS PHARM INC ;AARON DIAMOND AIDS RESEARCH CE (US)) 18 December 1997 (1997-12-18) abstract page 11, line 2 - line 33 page 12, line 3 - line 9 page 18, line 13 - line 15 page 21, line 9 - line 23 page 41, line 20 -page 42, line 9 page 49, line 24 -page 50, line 6 --- -/--	1,7-10, 12, 19-24, 26,27, 38,41, 42,48, 49,51

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 July 2000	24.08.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Montrone, M

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	BALTER M.: "Revealing HIV's T Cell Passkey" SCIENCE, vol. 280, 1998, pages 1833-1834, XP002143168 page 1833, column 3, paragraph 4 page 1834, column 1, paragraph 2 page 1834, column 3, paragraph 1 - paragraph 4 ---	1-55
Y	RIZZUTO C.D. ET AL: "A Conserved HIV gp120 Glycoprotein Structure Involved in Chemokine Receptor Binding" SCIENCE, vol. 280, 1998, pages 1949-1953, XP002143169 abstract page 1949, column 1, paragraph 1 -column 2, paragraph 2 page 1951, column 2, paragraph 2 -column 3, paragraph 1 page 1953, column 1, paragraph 2 ---	1-55
A	IDZIOREK T ET AL: "CONSTRUCTION OF CD4-BASED CHIMERIC MOLECULES BY CHEMICAL CROSS-LINKING" AIDS RESEARCH AND HUMAN RETROVIRUSES 1991, vol. 7, no. 6, 1991, pages 529-536, XP000916290 ISSN: 0889-2229 abstract page 530, column 1, paragraph 6 page 532, column 1, paragraph 2 page 533, column 1, paragraph 4 -page 534, column 1, paragraph 1 page 534, column 2, paragraph 3 -page 535, column 1, paragraph 1 --- -/--	1-55

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International Application No
PCT/US 00/06946

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	KWONG PETER D ET AL: "Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody." NATURE (LONDON) JUNE 18, 1998, vol. 393, no. 6686, 18 June 1998 (1998-06-18), pages 648-659, XP002143170 ISSN: 0028-0836 cited in the application abstract page 648, column 1, paragraph 2 -column 2, paragraph 1 page 654, column 2, paragraph 4 -page 655, column 2, paragraph 2 page 656, column 1, paragraph 3 -column 2, paragraph 2; figure 5 page 657, column 1, paragraph 2	1-55

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PC[redacted]S 00/06946

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9747318 A	18-12-1997	AU 3390297 A	07-01-1998
		AU 3402697 A	07-01-1998
		CA 2257991 A	18-12-1997
		EP 0956044 A	17-11-1999
		WO 9747319 A	18-12-1997

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
4239-54106		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/06946	16/03/2000	16/03/1999
International Patent Classification (IPC) or national classification and IPC C07K16/10		
Applicant THE GOVERNMENT OF THE UNITED STATES OF AME..et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02/10/2000	12.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Montrone, M Telephone No. +49 89 2399 8711

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/06946

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-34 as originally filed

Claims, No.:

1-55 as received on 13/03/2001 with letter of 13/03/2001

Drawings, sheets:

1/4-4/4 as originally filed

Sequence listing part of the description, pages:

1-5, filed with the letter of 08.06.2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 38,44-46,47,50, 51.

because:

☒ the said international application, or the said claims Nos. 44-46,47,50 with respect to IA relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 38, 51.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-37,39,40,44-47,50,52-55
	No:	Claims	41-43,48,49
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-37,39-50,52-55
Industrial applicability (IA)	Yes:	Claims	1-37,39-43,48,49,52-55
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Reference is made to the following documents:

D1: WO-A-9747318
D2: Science, vol. 280, 1998, p.:1833-1834
D3: Science, vol. 280, 1998, p.: 1949-1953
D4: AIDS RES. and HUMAN RETROVIRUSES, vol. 7, 1991, p.: 529-536

Item III:

Claims 47, 50 (completely) and claims 44-46 (partially) relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(i) PCT).

The subject-matter of claims 38 and 51 has not been searched. Thus, an opinion regarding novelty, inventiveness and industrial applicability of said claims cannot be formulated.

Item V:

1. Claim 1 relates to a neutralising bispecific fusion protein capable of binding to two sites on a target protein, comprising a first binding domain capable of binding to an inducing site on the target protein and a second binding domain capable of forming a neutralising complex with an induced epitope on the target protein and a linker connecting the first domain to the second domain, wherein at least one of the first and second binding domains comprises a binding portion of an antibody heavy or light chain.

   Such a protein is not known from the available prior art. Thus, the subject-matter of claim 1 is considered to be novel and complies with the provisions of Article 33(2) PCT. The same applies for the subject-matter of claims 2 to 32 being dependent thereon, the protein of claim 33 and nucleic acid sequences thereof (claims 34 to 37, 39), the cells of claim 40, the methods of claims 44 to 47, 50 and the kit of claims 52 to 55.

However, the methods of claims 41 to 43 and the protein of claims 48 and 49 are not considered to be novel for the following reasons:

D1 discloses a fusion protein consisting of a portion of the chemokine receptor C-C CKR-5 (CCR5) and the CD4 molecule for inhibiting an HIV-1 infection (see abstract, page 11, line 3 to page 12, line 9). Further chemokine receptors are mentioned, such as CXCR4, CCR3 or CCR-2b (see page 18, line 15). D1 also describes experiments which show an increased binding affinity of the CCR-5 to gp120 after a previous gp120-CD4 interaction (see page 49, line 24 to page 50, line 6). In addition, antibodies 48d and 17b are mentioned which bind to CD4-induced epitopes and which are considered to be identical to the antibodies referred to in claim 16. Furthermore, the region recognised by said antibodies is considered to be a prime candidate for the CCR5 binding region on the gp120 molecule and its known to be highly conserved (see page 50, line 21; page 51, lines 3 to 19 and page 52, lines 6 to 26). Since a portion of the chemokine receptor C-C CKR-5 (CCR5) is fused to the CD4 molecule which generally refers to "recombinantly fused" and the description teaches the recombinant production of CD4 and gp120 (see page 38, line 21 to page 40, line 13), D1 is still considered to be detrimental to the novelty of the subject-matter of claims 41 to 43. Moreover, said fusion protein is considered to be detrimental to the novelty of the compositions of claims 48 and 49 because it is considered to be an "analog" or "mimetic" of the fusion protein of claim 1.

Consequently, claims 41 to 43, 48 and 49 are therefore not considered to be novel and do not comply with the provisions of Article 33(2) PCT.

2. Moreover, the subject-matter of claims 1 to 37, 39, 40, 44 to 47, 50 and 52 to 55 appears not to be inventive for the following reasons:

D1 is considered as closest prior art. Said document already discloses a fusion protein which binds to two sites on a target protein wherein the first binding domain induces an epitope on said target protein and the second domain neutralises said induced epitope. The subject-matter of claim 1, 33, 40, 44 to 47 and 52 is distinguished therefrom by using a variable region of an antibody heavy or light chain instead of a fragment of the CCR-5 receptor. This difference results in an alternative

approach to inhibit for example an HIV-1 infection into CD4 positive T-cells.

The objective problem to be solved by the present application was therefore to find an alternative approach for neutralising an induced epitope.

However, D2 already suggested an approach wherein a second antibody, such as the single chain antibody 17b, would neutralise the CD4-induced epitope on the gp120 molecule for inhibiting an HIV-1 infection (see page 1833, right col., 4th para.; page 1834, left col., last para. to middle col., first para. and right col., first para. to third para.). D1 teaches as well that the 17b and 48d inhibit the gp120-CCR5 interaction and that this blockage may be particularly important (see page 50, line 22 and page 51, line 18, 19). Furthermore, 17b and 48d bind only to the conserved "CD4-induced epitope", meaning that the presence of CD4 is obligatory for the binding. In addition, D3 which describes a conserved HIV gp120 structure being involved in chemokine receptor binding after the prior binding of CD4 hints to the solution of the problem mentioned above by referring to inhibitors to CD4-bound states of the gp120 molecule (see abstract and page 1953, left col., last para.). Moreover, the existence of CD4-antibody chimeric molecules was known in the art. D4 discloses said fusion proteins by linking an anti-CD3 antibody to a soluble CD4 molecule which does not change the binding specificity of the two molecules (see abstract, page 533, 4th para. and page 534, right col., third para. to page 535, left col., first para.). The use of an additional linker as referred to in present claim 1 is considered to be a mere standard feature in the field of recombinant technology which does not result in any unexpected or non-obvious technical effect. Consequently, the underlying concept of the invention was already known . Thus, the person skilled in the art would have combined the teaching of D1 with D2 or D3 in order to solve the problem mentioned above and would have arrived at the claimed subject-matter falling within the scope of claim 1, 33, 40, 44 to 47 and 52 without employing any inventive skill. Consequently, the subject-matter of present claims 1, 33, 40, 44 to 47 and 52 does not appear to be inventive and does not fulfil the requirement of Article 33(3) PCT.

The dependent claims 2 to 32, 34-37, 39, 50 and 53 to 55 do not appear to contain any additional features which, in combination with the feature of the claim(s) to which they refer, involve an inventive step. Thus, said claims cannot be accepted either

under Article 33(3) PCT.

3. For the assessment of the present claims 47, 50 (completely) and claims 44-46 (partially) on the question whether they are industrially applicable, no unified criteria exist in the PCT. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Item VII:

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1-D4 is not mentioned in the description, nor are these documents identified therein.

Item VIII:

1. Claims 44 to 49 do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The following functional statements do not enable the skilled person to determine which technical features are necessary to perform the stated functions: "a variant protein, analog or mimetic thereof".

2. The scope of claim 36 is unclear since SEQ ID NO: 3 refers to an amino acid sequence and not to a nucleic acid sequence, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

3. Moreover, the scope of claim 52 is unclear since it refers to the generalised protein of claim 1 which does not allow any inhibition of an HIV infection (Article 6 PCT). Thus, the claim should rather refer to the specific protein of claim 33 which allows an inhibition of the HIV infection.

4. The single chain antibodies "SCFv17b, SCFv48d, SCFvCG10" as referred to in claim

16 and the corresponding monoclonal antibodies of claim 18 are considered to be mere internal designations without any technical information, rendering the subject-matter of said claims unclear (Art. 6 PCT).

5. Claims 44 to 47 refer back to claim 9. However, claim 9 refers to the target protein gp120 itself. This renders the scope and the meaning of said claims unclear, contrary to the provisions of Art. 6 PCT.

6. The term "A kit for treatment" as referred to in present claim 52 is considered to be unclear because "kits" are not administered to patients (Art. 6 PCT). It appears that the "kit" in the present case is rather a pharmaceutical composition.

We claim:

1. A neutralizing bispecific fusion protein capable of binding to two sites on a target protein, comprising a first binding domain capable of binding to an inducing site on the target protein, thereby exposing an induced epitope; a second binding domain capable of forming a neutralizing complex with an induced epitope of the target protein; and a linker connecting the first domain to the second domain.

2. A protein according to claim 1, wherein the first binding domain comprises a binding portion of a variable region of an antibody heavy or light chain.

3. A protein according to claim 2, wherein the first binding domain comprises an epitope binding domain of an antibody.

4. A protein according to claim 3, wherein the first domain comprises a single-chain Fv (SCFv).

5. A protein according to claim 3, wherein the antibody binding domain mimics a biological activity of a CD4 molecule in binding to the inducing site and exposing the inducing epitope.

6. A protein according to claim 5, wherein the antibody binding domain is derived from a CD4 anti-idiotypic antibody.

7. A protein according to claim 1, wherein the target protein is a viral envelope protein of a virus.

8. A protein according to claim 7, wherein the virus is human immunodeficiency virus (HIV).

9. A protein according to claim 8, wherein the viral envelope protein is gp120.

10. A protein according to claim 2, wherein the first binding domain is derived from a CD4 molecule.

11. A protein according to claim 10, wherein the first binding domain comprises CD4 D1 or CD4D1D2.

12. A protein according to claim 11, wherein the first binding domain is sCD4.

13. A protein according to claim 1, wherein the second binding domain comprises a binding portion of a variable region of an antibody heavy or light chain.

14. A protein according to claim 13, wherein the second binding domain comprises a binding domain of an antibody.

15. A protein according to claim 14, wherein the second binding domain comprises a single-chain Fv (SCFv).

16. A protein according to claim 15, wherein the SCFv is selected from the group consisting of SCFv17b, SCFv48d and SCFvCG10.

17. A protein according to claim 14, wherein the antibody binding domain is derived from a neutralizing monoclonal antibody.

18. A protein according to claim 17, wherein the neutralizing monoclonal antibody is selected from the group consisting of 17b, 48d, and CG10.

19. A protein according to claim 9, wherein the second binding domain mimics a biological activity of an HIV coreceptor molecule in binding to gp120.

20. A protein according to claim 19, wherein the second binding domain comprises a peptide fragment of an HIV coreceptor.

21. A protein according to claim 20, wherein the HIV coreceptor is a chemokine receptor.

22. A protein according to claim 21, wherein the chemokine receptor is selected from the group consisting of CXCR4, CCR2B, CCR3, and CCR5, CCR8, CCR9, CX_3CR1, US28, or the chemokine receptor related proteins including STRL33, GPR15, APJ, ChemR23, and BLTR.

23. A protein according to claim 9, wherein the induced epitope comprises at least one coreceptor binding determinant of gp120.

24. A protein according to claim 9, wherein the inducing site is a gp120 CD4 binding site.

25. A protein according to claim 14, wherein the binding domain of the antibody is capable of binding to at least one coreceptor binding determinant of gp120.

26. A protein according to claim 1, wherein the linker maintains the second binding domain in binding proximity to the induced epitope when the first binding domain is bound to the inducing site.

27. A protein according to claim 26, wherein the linker is substantially flexible.

28. A protein according to claim 26, wherein the linker is 15-100 angstroms (Å) long.

29. A protein according to claim 26, wherein the linker is 10-100 amino acid residues in length.

30. A protein according to claim 26, wherein the linker comprises at least one occurrence of an amino acid sequence as represented by SEQ ID NO: 1.

31. A protein according to claim 1, wherein the linker comprises at least one occurrence of an amino acid sequence represented by SEQ ID NO: 1.

32. A protein according to claim 31, wherein the linker comprises an amino acid sequence represented by SEQ ID NO: 2.

33. A functional recombinant bispecific fusion protein capable of binding to two sites on gp120, comprising:

a) sCD4;

b) SCFv(17b); and

c) a linker of a length sufficient to maintain the SCFv(17b) in binding proximity an SCFv(17b) epitope when sCD4 is bound to gp120.

34. A protein according to claim 33, wherein the linker has an amino acid sequence as represented by SEQ ID NO: 2

35. An isolated nucleic acid molecule encoding a protein according to claim 34.

36. A nucleic acid molecule according to claim 35, wherein the nucleic acid sequence is represented by SEQ ID NO: 3.

37. A protein encoded for by the nucleic acid molecule according to claim 36.

38. An isolated nucleic acid molecule encoding a protein according to claim 1.

39. The protein according to claim 38, having amino acid sequence SEQ ID NO: 4.

40. A transgenic eukaryotic cell comprising the isolated nucleic acid molecule according to claim 38.

41. A method for producing in a eukaryotic cell a functional recombinant bispecific fusion protein capable of binding two sites on a target protein, comprising the steps of:

a) transfecting the eukaryotic cell with a recombinant nucleic acid molecule according to claim 38;

b) culturing the transfected eukaryotic cell under conditions that cause production of the protein; and

c) recovering the protein produced by the cultured eukaryotic cell.

42. The method of claim 41, wherein the eukaryotic cell is a mammalian cell.

43. The method of claim 41, wherein recovering the protein comprises:

a) identifying the protein by the presence of a molecular tag; and

b) separating the protein having the molecular tag so identified from molecules without the tag, so as to recover the protein produced by the cultured eukaryotic cell.

44. A method for inactivating a gp120 protein, comprising the step of:

contacting the gp120 protein with a protein according to claim 9, or a variant protein, analog or mimetic thereof.

45. A method for neutralizing a human immunodeficiency virus, comprising the step of:

contacting the human immunodeficiency virus with a protein according to claim 9, or a variant protein, analog or mimetic thereof.

46. A method for blocking and preventing the binding of a viral or recombinant gp120 protein to soluble CD4 or lymphocyte CD4, comprising the step of:

contacting the gp120 protein with a protein according to claim 9, or a variant protein, analog or mimetic thereof.

47. A method for inhibiting HIV virus replication or infectivity in a subject, comprising administering to the subject an amount of the protein according to claim 9, or a variant protein, analog or mimetic thereof, sufficient to inhibit HIV virus replication or infectivity.

48. A composition comprising the protein according to claim 1, or a variant protein, analog or mimetic thereof.

49. A pharmaceutical composition comprising the protein according to claim 1, or a variant protein, analog or mimetic thereof, and a pharmaceutically acceptable carrier.

50. The method according to claim 47, wherein the protein is administered in the form of a pharmaceutical composition.

51. A protein analog, derivative, or mimetic of the protein of claim 1.

52. A kit for treatment and/or prevention of HIV infection, comprising a clinically effective dose of the neutralizing bispecific fusion protein of claim 1.

53. The kit of claim 52, further comprising instructions.

54. The kit of claim 53, wherein the instructions include directions for administering at least one dose of the neutralizing bispecific fusion protein to a subject in need of such treatment.

55. The kit of claim 52, wherein the neutralizing bispecific fusion protein is provided in the form of a pharmaceutical composition

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C07K 16/10, 16/42, 14/705, 14/715, 19/00, C12N 15/62, 5/10, A61K 39/42, 38/17, A61P 31/18 // C07K 16/28

A1

(11) International Publication Number: WO 00/55207

(43) International Publication Date: 21 September 2000 (21.09.00)

(21) International Application Number: PCT/US00/06946

(22) International Filing Date: 16 March 2000 (16.03.00)

(30) Priority Data:
60/124,681 16 March 1999 (16.03.99) US

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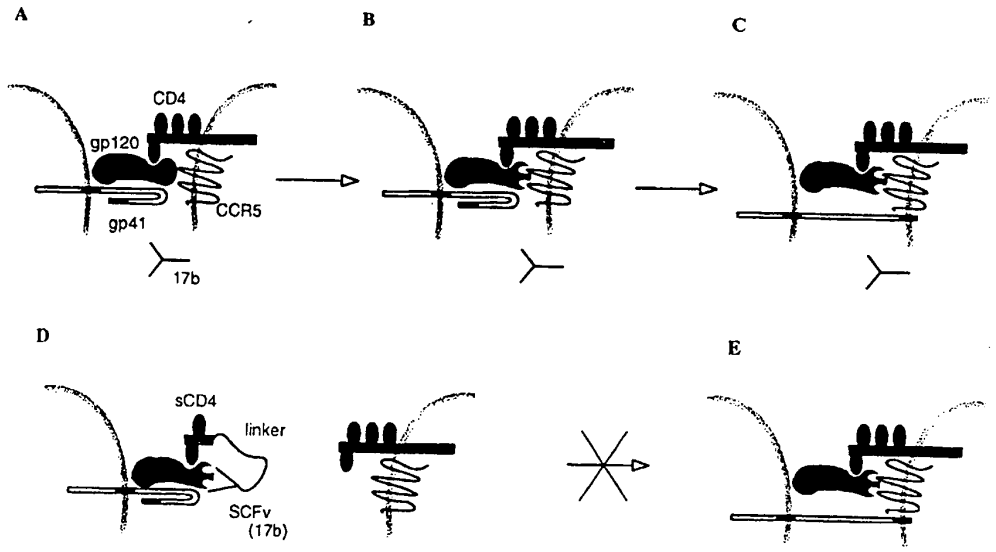
(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: A NOVEL CHIMERIC PROTEIN FOR PREVENTION AND TREATMENT OF HIV INFECTION



(57) Abstract

This invention relates to bispecific fusion proteins effective in viral neutralization. More specifically, such proteins have two different binding domains, an inducing–binding domain and an induced–binding domain, functionally linked by a peptide linker. Such proteins, nucleic acid molecules encoding them, and their production and use in preventing or treating viral infections are provided. One prototypical bispecific fusion protein is sCD4–SCFv(17b), in which a soluble CD4 fragment (containing domains D1 and D2) is fused to a single chain Fv portion of antibody 17b via a linker.

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INTERNATIONAL SEARCH REPORT

Inte. onal Application No
PCT/US 00/06946

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K16/10 C07K16/42 C07K14/705 C07K14/715 C07K19/00
C12N15/62 C12N5/10 A61K39/42 A61K38/17 A61P31/18
//C07K16/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, WPI Data, PAJ, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 47318 A (PROGENICS PHARM INC ;AARON DIAMOND AIDS RESEARCH CE (US)) 18 December 1997 (1997-12-18) abstract page 11, line 2 - line 33 page 12, line 3 - line 9 page 18, line 13 - line 15 page 21, line 9 - line 23 page 41, line 20 -page 42, line 9 page 49, line 24 -page 50, line 6 --- -/--	1,7-10, 12, 19-24, 26,27, 38,41, 42,48, 49,51

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
31 July 2000	24.08.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Montrone, M

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	BALTER M.: "Revealing HIV's T Cell Passkey" SCIENCE, vol. 280, 1998, pages 1833-1834, XP002143168 page 1833, column 3, paragraph 4 page 1834, column 1, paragraph 2 page 1834, column 3, paragraph 1 - paragraph 4 ---	1-55
Y	RIZZUTO C.D. ET AL: "A Conserved HIV gp120 Glycoprotein Structure Involved in Chemokine Receptor Binding" SCIENCE, vol. 280, 1998, pages 1949-1953, XP002143169 abstract page 1949, column 1, paragraph 1 -column 2, paragraph 2 page 1951, column 2, paragraph 2 -column 3, paragraph 1 page 1953, column 1, paragraph 2 ---	1-55
A	IDZIOREK T ET AL: "CONSTRUCTION OF CD4-BASED CHIMERIC MOLECULES BY CHEMICAL CROSS-LINKING" AIDS RESEARCH AND HUMAN RETROVIRUSES 1991, vol. 7, no. 6, 1991, pages 529-536, XP000916290 ISSN: 0889-2229 abstract page 530, column 1, paragraph 6 page 532, column 1, paragraph 2 page 533, column 1, paragraph 4 -page 534, column 1, paragraph 1 page 534, column 2, paragraph 3 -page 535, column 1, paragraph 1 --- -/--	1-55

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KWONG PETER D ET AL: "Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody." NATURE (LONDON) JUNE 18, 1998, vol. 393, no. 6686, 18 June 1998 (1998-06-18), pages 648-659, XP002143170 ISSN: 0028-0836 cited in the application abstract page 648, column 1, paragraph 2 -column 2, paragraph 1 page 654, column 2, paragraph 4 -page 655, column 2, paragraph 2 page 656, column 1, paragraph 3 -column 2, paragraph 2; figure 5 page 657, column 1, paragraph 2 -----	1-55

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/06946

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 47,50,52-55 (completely) and claims 44-46 (partially) are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 38, 51
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 38, 51

Present claims 38 and 51 relate to a product defined by reference to a desirable characteristic or property, namely a nucleic acid encoding all bispecific fusion proteins or all protein analogs, derivatives or mimetics being capable of binding to two sites on a target protein wherein the first binding domain binds to an inducing site on the target protein and the second binding site neutralises said induced epitope by forming a complex. The two domains are linked by a linker.

The claims cover all products having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such products. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/06946

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9747318 A	18-12-1997	AU 3390297 A	07-01-1998
		AU 3402697 A	07-01-1998
		CA 2257991 A	18-12-1997
		EP 0956044 A	17-11-1999
		WO 9747319 A	18-12-1997